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Abstract: AIM Citric acid (CA) is a component in beverages responsible for dental erosion. The aim of this study was to examine the influence of CA with different pH, titratable acid and buffer capacity (β), and the impact of the chelating effect of CA on development of enamel erosions. **MATERIAL AND METHODS** In a superfusion model, hydroxy apatite (HAp) dissolution of bovine enamel was measured in four experiments (EXP 1-4) with 27 experimental groups ($n = 8$ per group). The samples were superfused with different CA variations and respective controls. EXP-1: Dilution series of HCl (pH 2.15-3.02). EXP-2: Dilution series of natural CA (56-1.75 mmol l(-1); pH 2.15-3.02). EXP-3: CA solutions (56 and 14 mmol l(-1), β : 39.7 and 10.2 mmol l(-1) pH(-1), respectively) with different titratable acidity at equal pH values. EXP-4: CA concentrations (56-1.75 mmol l(-1)) neutralized to pH 7. **RESULTS** CA led to higher HAp-dissolution than HCl. With higher pH, the difference in HAp-dissolution rate between the two acids became increasingly smaller. At equal pH, HAp-dissolution was higher for the CA with the higher amount of titratable acid. However, no clear correlation between erosion and titratable acid or β could be found. Only minimal amounts of HAp were dissolved by neutralized CA compared to CA with natural pH. **CONCLUSION** Under the chosen conditions chelating effects of CA do not have a relevant influence of HAp-dissolution of enamel. Moreover, amount of HAp-dissolution by CA is not attributed to a single factor alone. The interplay between the different parameters of CA seems to be responsible for its erosive potential. **CLINICAL RELEVANCE** The erosive potential of solutions containing citric acid with unknown concentrations could not be predicted using a single parameter alone, and should at best determined in experimental set-ups.

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Chelating effect of citric acid is negligible for development of enamel erosions.

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Abstract

Aim: Citric acid (CA) is a component in beverages responsible for dental erosion. The aim of this study was to examine the influence of CA with different pH, titratable acid and buffer capacity (β), and the impact of the chelating effect of CA on development of enamel erosions.

Material and Methods: In a superfusion model, hydroxy apatite (HAp) dissolution of bovine enamel was measured in four experiments (EXP 1-4) with 27 experimental groups (n=8 per group). The samples were superfused with different CA variations and respective controls. EXP-1: Dilution series of HCl (pH 2.15–3.02). EXP-2: Dilution series of natural CA (56–1.75 mmol l⁻¹; pH 2.15–3.02). EXP-3: CA solutions (56 and 14 mmol l⁻¹, β : 39.7 and 10.2 mmol l⁻¹ pH⁻¹, respectively) with different titratable acidity at equal pH values. EXP-4: CA concentrations (56–1.75 mmol l⁻¹) neutralized to pH 7.

Results: CA led to higher HAp-dissolution than HCl. With higher pH, the difference in HAp-dissolution rate between the two acids became increasingly smaller. At equal pH, HAp-dissolution was higher for the CA with the higher amount of titratable acid. However, no clear correlation between erosion and titratable acid or β could be found. Only minimal amounts of HAp were dissolved by neutralized CA compared to CA with natural pH.

Conclusion: Under the chosen conditions chelating effects of CA do not have a relevant influence of HAp-dissolution of enamel. Moreover, amount of HAp-dissolution by CA is not attributed to a single factor alone. The interplay between the different parameters of CA seems to be responsible for its erosive potential.

Clinical relevance: The erosive potential of solutions containing citric acid with unknown concentrations could not be predicted using a single parameter alone, and should at best determined in experimental set-ups.

Introduction

Dental erosion is a process of demineralization resulting in surface-softening and irreversible loss of hard tissue induced by acidic compounds in an environment undersaturated with respect to tooth mineral [1]. It is suggested that beside the pH of an acidic solution, many other factors are related to the capacity of a solution to induce dental erosion, such as acid type and concentration, titratable acidity, buffering capacity and un-dissociated acid concentration [2]. Among other acids, citric acid (Cit) is very common in beverages and known for its erosive effect and its ability to form complexes with calcium dissolved from the dental crystals. Different complexes with different dissociation constants (pK_{Ca}) may be formed, such as ($Ca^{2+} + H_3Cit^- \rightleftharpoons CaH_3Cit^+$, $pK: 1.10$), ($Ca^{2+} + H_2Cit^{2-} \rightleftharpoons CaH_2Cit$, $pK: 3.09$), ($Ca^{2+} + HCit^{3-} \rightleftharpoons CaHCit^-$, $pK: 4.68$), ($Ca^{2+} + 2H_2Cit^{2-} \rightleftharpoons Ca(H_2Cit)_2^{2-}$, $pK: 6.79$), ($Ca^{2+} + 2HCit^{3-} \rightleftharpoons Ca(HCit)_2^{4-}$, $pK: 8.02$) [3,4]. Larger pK -values indicate stronger binding of the ligand to calcium [3]. This formation of calcium complexes by citrate molecules is indicated as an additional relevant factor for the loss of dental hard during erosion. According to a recent publication by Shellis et al. [3], the extent to which this phenomenon enhances the erosive effect of organic acids has yet to be quantified. It is obvious that citric acid is a very complex and specific chemical compound rendering it difficult to predict the erosive potential of differently concentrated citric acid solutions.

Citric acid has been applied in many erosion studies using different concentrations and different experimental set-ups with various analytical methods used for measuring the erosive effect [5,6].

However, up to now, no systematic experiment has been performed to elucidate the specific properties of citric acid and their impact on dental erosion.

Therefore, the aim of this study was to examine systematically the properties mentioned above as well as the influence of the chelating effect of citric acid on the development of enamel erosions.

Material and methods

As a first step, the characteristics of natural citric acid were analysed. Therefore, 10.76 g citric acid (Fluka Nr.27488, Buchs, Switzerland) were dissolved in 1000 ml

distilled water. This stock solution with 56 mmol l^{-1} citric acid was used for a geometric dilution series (Table 1). For this, one part of the previous concentration was collated with the same volume of distilled water. The final pH-values were between 2.17 and 3.02, respectively. Titration curves were measured for all acid dilutions. For that purpose, NaOH (Titrisol NaOH 1 mol/l ; Merck, Darmstadt, Germany) was successively added to the acid, using a titrator (Metrohm, Herisau, Switzerland). The change of pH, as well as the OH^- -addition were registered as titration curve. The titratable acid was calculated from the respective titration curve up to pH 7. All titration curves ran linear far over pH 5.5. The amount of OH^- , which neutralizes the acid about one pH unit, is called buffer capacity β . It corresponds to the slope of the respective titration curve.

The solutions were prepared immediately before being used in the respective experiment (1-4), in which enamel samples were superfused with the acidic solution.

Preparation of enamel specimens

Extracted bovine teeth, which were preserved in 0.1% thymol solution, were used for preparation of enamel specimens. Then, 216 enamel cylindrical specimens with a diameter of 3 mm were prepared using a diamond trephine mill. They were embedded in acrylic resin blocks (6 mm in diameter, Paladur, Heraeus Kulzer GmbH, Hanau, Germany). All specimens were ground flat with water-cooled carborundum discs (1500, 2500 and 4000 grit, Water Proof Silicon Carbide Paper, Stuers, Erkrath, Germany).

Rationale behind and description of experiments 1-4

- Experiment 1 (EXP-1): For dissolution of a hydroxy apatite molecule (HAp) a constant amount of protons is needed. Therefore a strong correlation between erosion and activity of protons in a solution can be assumed.

With the first experiment, the correlation of the enamel dissolution with the initial H^+ -activity should be investigated. Hydrochloric acid (Titrisol HCl, Merck, Darmstadt, Germany) was diluted with distilled water to the same pH-values of the geometric dilution series of citric acid (Table 2).

As described later, enamel samples were superfused with the HCl-dilutions. The HAp dissolution was determined by measuring Ca^{2+} -release from the enamel surface. Hydrochloric acid is almost completely dissociated. Thus, the titratable acid of HCl is

identical with the proton activity (H^+ , mmol l^{-1} , see Table 2) of the solution. This means that the degree of erosion can be attributed directly to the proton activity and the respective pH.

- Experiment 2 (EXP-2): PH-value of an acid shows the current H^+ -activity. With citric acid, only a small part of the acid is dissociated. Consumed protons will be immediately delivered by dissociation of further citric acid molecules.

The aim of the second experiment was to analyse the influence of pH-value and H^+ -activity of citric acid on the erosion. A geometric dilution series of natural citric acid and H_2O was prepared. Enamel samples were superfused with these solutions with pH-values between 2.17 and 3.02 (Table 1).

- Experiment 3 (EXP-3): It might be assumed that citric acid in beverages is often partially neutralized. Thus, the titratable acidity and pH of beverages may deviate from the values of the natural citric acid. Consumed protons will be additionally delivered by dissociation. This experiment was done to analyse the correlation between titratable acidity of citric acid and its erosive capacity.

Two citric acid stock solutions (56 and 14 mmol l^{-1}) were prepared. Parts of these solutions were adjusted with NaOH (1 mol l^{-1}) or HCl (1 mol l^{-1}) to pH-values ranging from 2.17 to 7.0. As a result, two series of acids with equal pH but different titratable acid were obtained (Table 3).

It was suggested that the erosion rate of enamel superfused with solutions of the same pH should be mainly dependent on the different titratable acid.

- Experiment 4 (EXP-4): In a previous study, citric acid dissolved more enamel than HCl at the same initial pH during superfusion [7]. The question remained open, if chelation processes of calcium with the citric acid molecule were relevant for this.

This experiment was designed to evaluate, how much calcium might be maximally dissolved by chelation only of enamel during superfusion. Therefore, citric acid dilutions of the second experiment were neutralized with NaOH to pH 7. This results in CA solutions with 56, 28, 14, 7 and 3.5 mmol l^{-1} , respectively.

Enamel samples were superfused with these neutralized citric acid solutions. Due to the lack of protons, it should be assumed that no acid-related erosion might occur.

Thus, it was supposed that the calcium release of the enamel should be caused by chelation only.

Experimental procedures

The 216 enamel specimens were randomly allocated to 27 groups ($n = 8$ per group). The samples were fixed in superfusion chambers described in detail previously [7]. Erosive solutions were pumped with an eight channel peristaltic pump for 15 min. Each superfusion chamber was loaded with a single enamel sample. The erosive solution flowed over the whole enamel surface of 7.07 mm^2 . The layer height of the acid was 0.3 mm. The pump output was $133 \mu\text{l min}^{-1}$, the resulting flow rate was 2.47 mm s^{-1} . After the passage, the acidic solution were caught and analysed with regard to their calcium and phosphate contents.

The released calcium was determined by atomic absorption spectroscopy (AAS) allowing for measuring ionized calcium in the solutions. Chelated calcium was not quantified. In pilot tests showed with AAS is a very sensitive method, but requires a relatively large test volume. Since in the superfusion experiments only 2 ml of acid were available, the method had to be modified. Pilot experiments had shown that measurements of a sample started to be stable after the third consecutive measurement. Thus, three measurements were made in series of the standards and the samples, of which only the third determination was used for calculation. A new calibration was performed after every 20 samples. The calcium concentrations of the samples were calculated with a dose-response curve using the mean values of the standards measured. Curve-fitting with a second order polynomial allowed the quantification of our samples with very low calcium concentration.

To check the plausibility of the measured calcium values, phosphate analysis was performed using a colorimetric analysis with malachite green/ammonium molybdate [8,9]. Absorption was measured after 8 min, due to instability of the colour complex [10].

Further analysis of recorded data

Calcium dissolution was transformed to the erosion rate expressed as dissolution of hydroxy apatite [$\mu\text{mol HAp s}^{-1} \text{ m}^{-2}$] and loss of bovine enamel [$\mu\text{m min}^{-1}$], according

to the equations in Figure 1. This calculation was done, in order to allow comparison of the findings of the present study with results of other publications.

Moreover, the amount of consumed and un-dissociated acid, respectively, was calculated for the HAp-dissolution in the applied pH-range as described recently [11]: $14 \text{ H}^+ + \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \rightarrow 10 \text{ Ca}^{2+} + 6 \text{ H}_2\text{PO}_4 + 2 \text{ H}_2\text{O}$. For 10 nmol of dissolved Ca, a consumption of 14 nmol H^+ was assumed.

Statistical analysis

Regression analysis and correlation coefficients were calculated in all experiments. The descriptive statistics and regression analysis were computed with the statistical program SPSS 20.0.0.2. performed. Significances of the correlations have been tested for the 1% level. Results of the statistical analysis with p-values below 0.01 were considered to be statistically significant.

Results

The results of the analysis of the properties and effects of CA and the different experiments are given in the Figures 1-7. For all correlations, the curves fitted were highly significant ($p < 0.001$).

Buffer capacity (β) of the CA solutions with concentration of 14 and 56 mmol/l, respectively, is demonstrated in Fig. 2. Buffer capacity, as calculated from the titration curves of citric acid solutions, is presented in figure 3. It is evident that each citric acid concentration comes up with a specific buffer capacity in the pH range used in the experiments. The titration curves of all citric acid solutions used in these study showed a typical buffer capacity during the whole experiment. The respective titration curves ran linear, far over the pH 5.5-value. The outcome of this was a constant β -value for every citric acid concentration.

- EXP-1: In Figure 4, the HAp-dissolution of enamel samples superfused with HCl is shown as function of the H^+ -activity. A strictly linear correlation between HAp-dissolution and the initial proton activity of HCl was observed.

- EXP-2: HAp-dissolution of enamel samples superfused with citric acid is shown as function of the H^+ -activity is given in Figure 5. The erosion rate of citric acid slightly

increased with the rise of the H^+ -activity. At the same initial pH, the diluted citric acid dissolved more hydroxy apatite than HCl in EXP-1.

In Figure 6, the association between pH and HAp-dissolution of citric acid or hydrochloric acid is presented. The values are taken from the results of EXP-1 and EXP-2. No linear correlation between pH and HAp-dissolution was found for both acids. At all pH-levels, citric acid led to higher HAp-dissolution of the enamel specimens than the hydrochloric acid. With higher pH-levels, the difference in HAp-dissolution rate between the two acids became increasingly smaller.

- EXP-3: Figure 7 shows the association between titratable acid and HAp dissolution. Two different concentrations of citric acid (14 and 56 mmol/l) have been adjusted with NaOH or HCl to the pH values of 2.17; 2.33; 2.51; 2.66; 2.84 and 3.02. The dissolution of the samples by superfusion is shown separately for each acid concentration, likewise the resulting solutions of different titratable acid levels. The titratable acid presented an exponential correlation to the erosion rate. For each respective acid concentration, no linear correlation between HAp-dissolution and titratable acid was indicated. Comparison of both citric acid concentrations (demonstrated by the broken line) showed that the same HAp-dissolution was caused by citric acid solutions with different amounts of titratable acid.

- EXP-4: Figure 8 presents the HAp-dissolution rate of citric acid solution with natural pH and neutralized citric acid at same concentrations. The HAp-dissolution values of the two lowest neutralized citric acid concentrations were in the range of measurement limit and could therefore not be determined reliably. The diagram shows that after neutralization of citric acid, only minimal amounts of HAp were dissolved, as compared to the amount of HAp dissolved by natural acid.

In Figure 9, the degree of dissociation and acid consumption (given as percentage of titratable acid) of different citric acid dilutions during superfusion is presented. The consumed acid was calculated with the dissolved calcium and refers to the acid used up for demineralisation in the superfusion chamber. The dashed line indicates 2/3, the dotted line 1/3 of the initial titratable acid. Acid dilutions with lower pH-values were less dissociated. After the superfusion experiments the consumed acid was

calculated via the calcium dissolution. Obviously, most of the acid remained undissociated. The percentage of consumed acid increased with the initial pH-value.

Figure 10 presents the HAp-dissolution rate of enamel induced by CA with buffer capacity (β) of 10.2 and 39.7 mmol l⁻¹, respectively, adjusted to different pH-values. Data were extracted from EXP 3. It is evident that CA with equal buffer capacity led to different HAp-dissolution despite differences in pH. Moreover it is obvious that with decreasing pH an increase in HAp-dissolution rate occurred for the citric acid with β = 39.7 mmol l⁻¹ pH⁻¹. This increase was less distinct for the citric acid with lower buffer capacity.

Discussion

Use of bovine enamel samples and citric acid in a superfusion model

In the present study, bovine enamel from teeth of cattles was used, as bovine teeth can be gained without any problems in homogenous quality. Their chemical and mechanical properties are similar to human dental hard tissues. The anatomy of bovine teeth is similar to human teeth [12,13]. Thus, bovine teeth are widely used in studies analysing the impact of various conditions on enamel erosions.

In literature, several chemical factors of solutions, such as pH, buffer capacity, titratable acid, type of acid, mineral contents and chelating activities are claimed to be responsible for the severity of erosive defects [14,15]. The influence of these factors on erosion and their relevance is always controversial discussed [11,16,17]. It is difficult to determine, which acidic characteristics are most important with regard to tooth erosion. In the present series of experiments, different acid characteristics were separately checked for their respective impact on the erosive potential. Thus, these different parameters are firstly discussed separately in the following. Indeed, many of these factors are dependent on each other. Thus, it was recently stated that titratable acidity to pH 5.5 is probably the preferred practical alternative method for measuring the contribution of buffering to erosive potential and that the nonlinear relationship of the dissolution rate to pH and to buffering properties need to be taken into account when multivariate models of erosive potential are constructed [17].

This study tried to analyse citric acid regarding these characteristics, as one of the most common acids in erosive beverages. Citric acid dissolved in water, is

dissociated only to a small part. This means that the protons are mainly bound by the citric acid molecules. The relation between bound and free protons is determined by acid-specific natural constants. The free protons are measurable and are stated as the negative logarithm of their activity (pH-value). These protons are responsible for erosion. If protons are consumed by erosion, further protons will be released, until a natural balance is re-established. The buffer capacity β shows, how many protons can be further dissociated, before their activity drops to 1/10. The titratable acid is measured as the maximum of protons, which are available for erosion. Buffer capacity and titratable acid are linear dependent on the concentration of natural citric acid. Citric acid has the capability to complex metallic ions, such as calcium, iron or magnesia. It should be noted that only citric acid molecules that have delivered at least two of its three protons, are able to bind Ca^{2+} in a complex. Thus, it should be realized that the pKa-values for citric acid are: $\text{pKa}_1 = 3.13$, $\text{pKa}_2 = 4.74$ and $\text{pKa}_3 = 6.42$. This means that complexation of calcium by the citric acid anion takes place preferably at $\text{pH} > 4$ [3].

The highest citric acid concentration used in the present study amounted to 56 mmol l^{-1} . This corresponds approximately to the concentration of citric acid in orange juice. In citric acid with 56 mmol l^{-1} about 30% of the acid is neutralized, so that a pH of 3.6 and titratable acidity of 120 mmol l^{-1} results [18]. In EXP-4 it was investigated, how much hydroxy apatite can be dissolved, if this citric acid is completely neutralized and is able to form a maximum amount of chelate complexes.

Usually, acid contact with the enamel during drinking of an acidic beverage and the respective pH-drop at the tooth surface is just limited to a few seconds [19,20]. Therefore, the experiments were performed on enamel samples, which were rinsed by the acidic solutions in a superfusion chamber. The applied citric acid was chosen in a pH-range of 2-3 typical for erosive beverages [21] [18].

Discussion of the results

The experiment with the dilution series of natural citric acid and HCl attended to the question, how erosion is related to the initially dissociated protons. The results obtained by using HCl confirm the clear linear correlation with the initial proton activity. During dissolution of one HAp-molecule, 10 Ca^{2+} -atoms are delivered and 14 H^+ are consumed [11]. PO_4 -molecules, which are also delivered, bound 1-2 protons,

depending on the pH-value. Hence it was calculated that 13-14% of the free protons were consumed for the HAp-dissolution during superfusion by every inserted HCl-dilution.

In literature, it is often attempted to correlate the erosion rate with the pH-value. If a linear dependency between the proton activity and the erosion rate was proved, a non-linear correlation between the negative logarithm of the H^+ -activity and HAp-dissolution is obligatory. EXP-1 and EXP-2 showed that for all applied acidic mixtures an exponential correlation between erosion rate and the initial pH-value was found. At the same pH-values, erosion rate for citric acid was higher than for HCl. This effect is assumed to be due to the immediate release of new protons from citric acid during superfusion. Thus, disproportional higher erosion with increasing concentration of citric acid was recorded. The higher the activity of un-dissociated protons, the faster is the release of consumed H^+ . The deviation from the linear correlation between the proton activity and erosion will grow, when the acid contact is prolonged.

The results of EXP-3 acid showed an exponential relation of the amount of titratable acid with the erosion, for every acid concentration applied in the experiment. Comparison of both citric acid concentrations indicated that the same erosion could be caused by different amounts of titratable acid. Thus the experiment shows, that a clear and linear correlation between the amount of titratable acid and the erosive capacity on enamel is not given for the solutions applied.

The experiments yielded that the buffer capacity β was linear proportional to the citric acid concentrations applied. It should be noted that a the linear titration curve of citric acid exists for a wide range of pH steps. However, as presented in figure 10, dissolution rate of hydroxy apatite was essentially higher at lower pH (e.g. 2.17) than at higher pH (e.g. 3.02) for the same buffer capacity. Strikingly, the experiments showed that no clear correlation between buffer capacity of citric acid solutions and the induced erosive effects is given. This means that various HAp-dissolution rates could be related to one β .

The influence of chelation was examined by using citric acid, neutralized by NaOH. Neutralization of citric acid leads to a solution with proton-free citric acid molecules.

This solution is supposed to form calcium-chelates easily. In other words, dissolution of enamel induced by neutralized citric acid is only driven by chelation of calcium dissolved from the enamel. EXP-4 showed that the neutralized citric acid was only able to dissolve hydroxy apatite in a range of about 2% as compared to the amount of hydroxy apatite dissolved by the natural, un-neutralized acid.

In a recent study West et al. [22] also investigated the influence of chelating effects of citric acid on enamel erosion was. In contrast to the present study, it was concluded that citric acid does have a chelating effect while eroding dental enamel. West et al.[22] neutralized citric acid (15.6 mmol l^{-1}) with NaOH to variable pH values, where a part of the citric acid is transformed to sodium citrate. That means that two H^{+} -ions react irreversibly with OH^{-} to H_2O and are lost for further reactions with hydroxy apatite. The substance loss was compared with the erosive effect of citric acid (15.6 mmol l^{-1}) mixed with increasing amounts of tri-sodium citrate without neutralising the protons. The latter solutions led to higher enamel substance loss than the neutralized citric acid. The authors attributed this effect to formation of chelate complexes. However, it should be considered that the pronounced substance loss in the solutions with tri-sodium citrate might also be caused by the higher amount of titratable acidity. Moreover it should be considered that in the study by West et al. [22] the samples were moved for 30 min in the same acidic solutions, whereas the enamel specimens in the present study were superfused with the acid in a flow model. Erosion is a concentration- and time-dependent chemical reaction. During 30 min even low concentrated protons may still react with hydroxy apatite. This could lead to a measurable dissolution of enamel with proton involvement. However, this is not the case in a fast passage of acidic beverages during drinking. Both, the concentration of the acids and the long contact time chosen in the study of West et al.[22], are not comparable with the situation during the consumption of erosive drinks.

Additionally, as shown in Figure 9, the amount of citric acid being present in an undissociated form after superfusion and contact with the enamel surfaces of the specimens, was in the present study higher than 82% for all citric acid solutions applied. That means, that even after superfusion most of the protons stayed bound to the citrate molecule rendering complex formation of calcium with the citrate molecule very unlikely to occur.

Summarized, it might be concluded that the differences in study design and citric acid preparation between the present study and the study conducted by West et al. [22] might be responsible for the difference in the estimation of the relevance of a chelating effect during citric acid induced enamel erosions. The present study proves that under flowing conditions a chelating effect of citric acid seems to be of negligible relevance with respect to enamel erosion.

In the present superfusion experiments, the flow rate for all acids was constant. The enamel surface with hydroxy apatite as substrate was standardized, so that reaction time and amount of substrate was equal in each sub-experiment. As mentioned above, HAp-dissolution by H^+ -ions is a first order reaction, where one molecule HAp reacts with 14 H^+ -ions. The reaction rate correlated linearly with the initial proton activity. This reaction reduces the concentration of protons in the solution and the pH increases. Thus, the initial equilibrium between the un-dissociated acid and free protons is disturbed, so that citric acid must release additional protons, until the natural relation between un-dissociated and dissociated acid is restored. This means that in, during dissolution of hydroxy apatite in an incubation model, the titratable acid is partially consumed. This is not the case, when a superfusion model is chosen, as in the present study.

As already described, the recorded calcium dissolution of the enamel was converted into hydroxy apatite dissolution rate and enamel loss. This allowed us to compare different studies with each other and to compare our results with data, which were obtained by using citric acid without further pH adjustment.

Citric acid can be present as pure acid or as a monohydrate, with a 9% higher molecular weight. Some authors did not specify, in which form the % (w/v)-solution was used and the exact molarity of the acid remains unclear.

This might be the reason for the observation that the specified pH value scatters considerably between different publications for the same acid concentration used. We measured pH 2.47 in 15.6 mmol l^{-1} citric acid (0.3%) solution, in accordance to other authors [23]. In contrast, in other studies this citric acid concentration was specified with pH 2.15 [24]. These differences should be kept in mind, when comparing the results of various studies, evaluating the effect of citric acid and enamel.

Also, the experimental designs of different studies are showing a great variability, which might be responsible for the variability of the effects of citric acid with identical concentrations. For example, using 24 mmol l⁻¹ citric acid in a reciprocating movement of 35 min⁻¹, Ganss and co-workers [25] recorded an enamel substance loss of 0.12 µm min⁻¹, which was five times less than the erosion rate resulting in our superfusion experiment using the same citric acid concentration. Additionally, in series of experiments applying 50 mmol l⁻¹ citric acid (pH 2.3) Schlueter et al. [26] recorded erosive effects in enamel between 0.24 and 0.45 µm min⁻¹, which was lower as compared to the HAp-dissolution rate (1.11 µm min⁻¹) measured in the present study for the same citric acid concentration. In another study, using 14 mmol l⁻¹ citric acid (pH 2.8) resulted in an enamel substance loss of 1.15 µm min⁻¹. [27] This effect was more than twice of the effect (0.43 µm min⁻¹) as measured in our experiment for the same citric acid concentration. Using calcium analyses, similar to that of the present study, Hemingway et al. [28] found an enamel dissolution rate with this acid of 2.5 nmol min⁻¹ mm⁻², whereas the present study showed a dissolution rate of 0.98 nmol min⁻¹ mm⁻². Finally, Shellis et al. [29] described a hydroxy apatite dissolution rate of 6.9 nmol min⁻¹ mm⁻² using 15.6 mmol l⁻¹ citric acid on dental enamel, thus 6 times higher as recorded in our experiments using the same citric acid concentration. Taking these comparisons together, it could be noted that the enamel dissolution rate observed in the present series of experiments are up to 5x higher or up 6x lower than reported in other studies using the same concentration of citric acid. This shows that comparison between the outcomes of different studies dealing with citric acid properties should be done with caution. In the present study, the erosive effect of citric acid could not be attributed to a single characteristic of the acid alone, such as chelating effect citric acid, buffer capacity and amount of titratable acid. For citric acid we found a nearly linear correlation only for the proton activity. Buffer capacity is dependent on acid concentration and the proton activity depends on acid concentration and titratable acid. At a first glance, this observation seems to be in contrast to some studies, reporting these properties to be relevant for prediction of enamel dissolution rate [17,29]. However, it was stated that the rate of dissolution of enamel is related to buffer capacity and titratable acidity, but that on the other side, the relationship to both variables is dependent on acid type and pH with a non-linear relationship of the buffering properties to enamel dissolution rate [17]. This underlines that complex multivariate models are necessary to predict enamel dissolution

properties of an acidic solution. Thus, prediction of erosivity of drinks with unknown citric acid concentrations or mixtures should be done with caution, showing the necessity of an experimental determination of the erosive potential of those solutions.

Conclusions

The results of the present experiments proved that chelating effects of citric acid have only a negligible impact on erosive strength of citric acid under the chosen conditions of an superfusion model. Moreover, in this model, neither the pH nor the titratable acidity and buffer capacity of citric acid show a linear correlation to enamel dissolution rate. However, for dilutions of natural citric acid an almost linear dependency of the erosion rate from H^+ -activity exists. Thus, erosivity of drinks with natural (not partially neutralized) citric acid might be estimated approximately by the initial proton activity. However, prediction of erosivity of drinks with unknown concentrations or citric acid mixtures by using a single parameter alone, such as pH, buffering capacity or titratable acidity, should be done with caution and is not able to replace an experimental determination.

Conflict of interest statement

The authors have no conflict of interest.

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Figures 1-9

$$\mu\text{mol HAp s}^{-1}\text{m}^{-2} = \frac{Ca (\mu\text{mol ml}^{-1}) V(\text{ml})}{A(\text{mm}^2) 10^6 t(\text{s}) 10} = Ca (\mu\text{mol ml}^{-1}) 31.43$$

with:

volume V = 2 ml

surface A = 7.07 mm²

time t = 15 min = 900 s

1 mol HAp = 10 mol Ca [3]

Fig. 1: Equation for transformation of calcium dissolution to erosion rate expressed as $\mu\text{mol HAp s}^{-1}\text{m}^{-2}$.

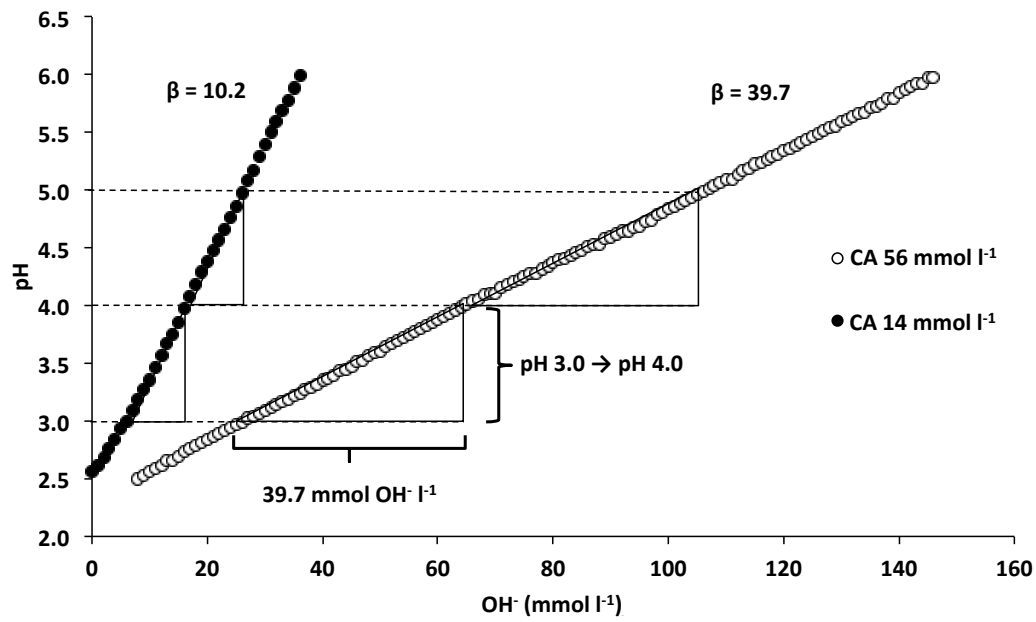


Fig. 2: Titration curves of CA (14 und 56 mmol l⁻¹) taken from the measurements during mixing of CA variations. Buffer capacity (β) is defined as OH⁻ used to change the pH value for one unit.

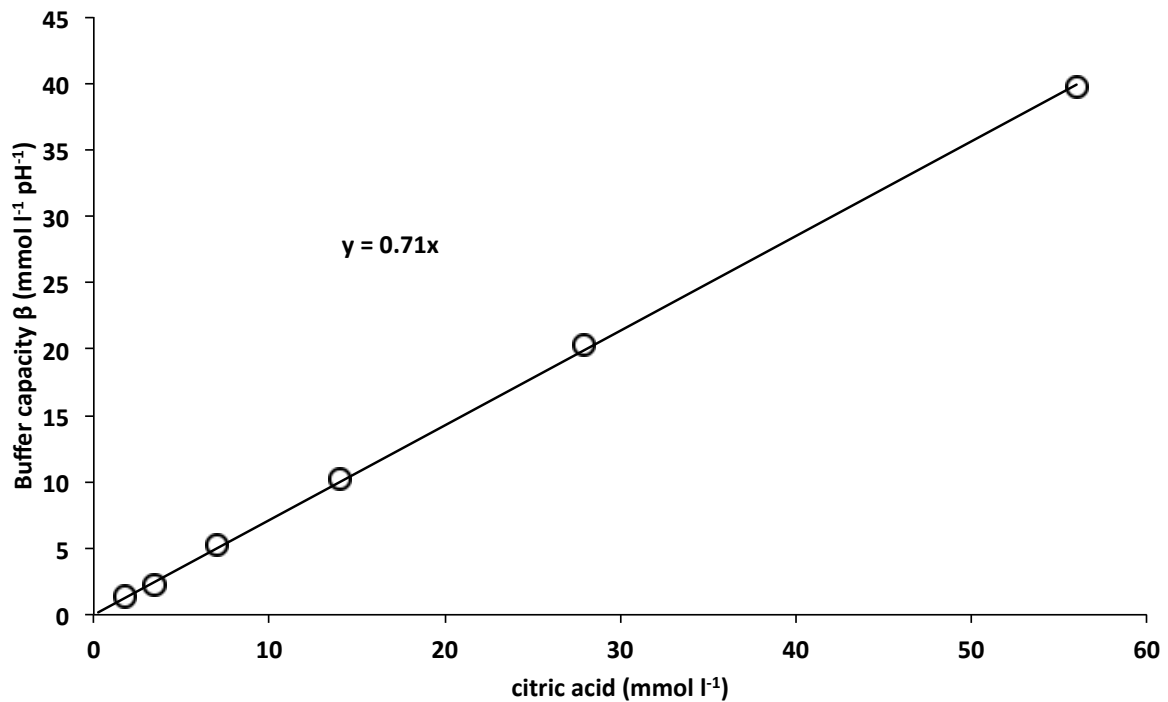


Figure 3: Buffer capacity β calculated from the titration curves of citric acid solutions.

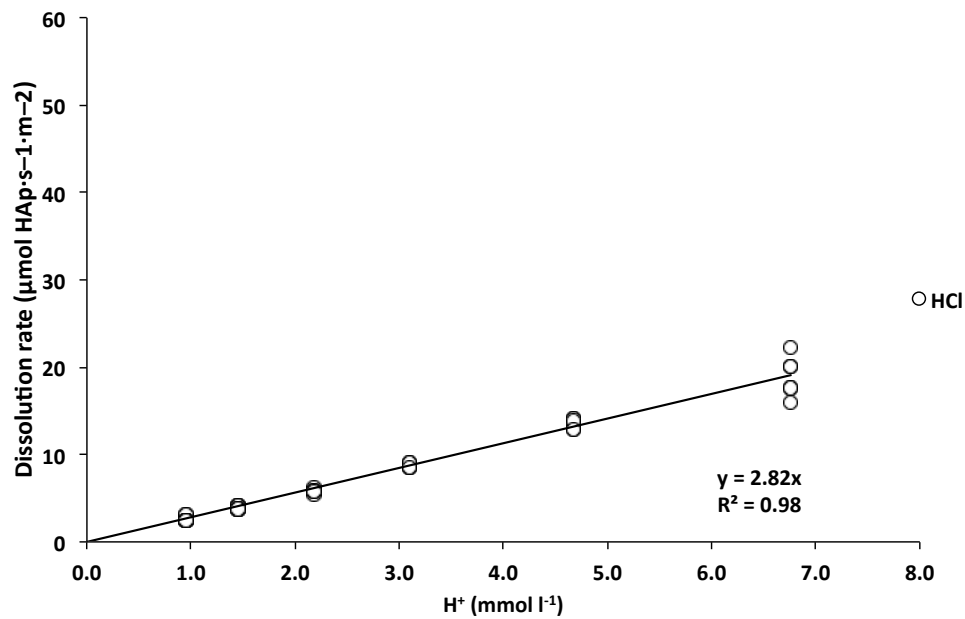


Figure 4: HAp-dissolution of enamel samples superfused HCl dilutions as function of initial H^+ -activity.

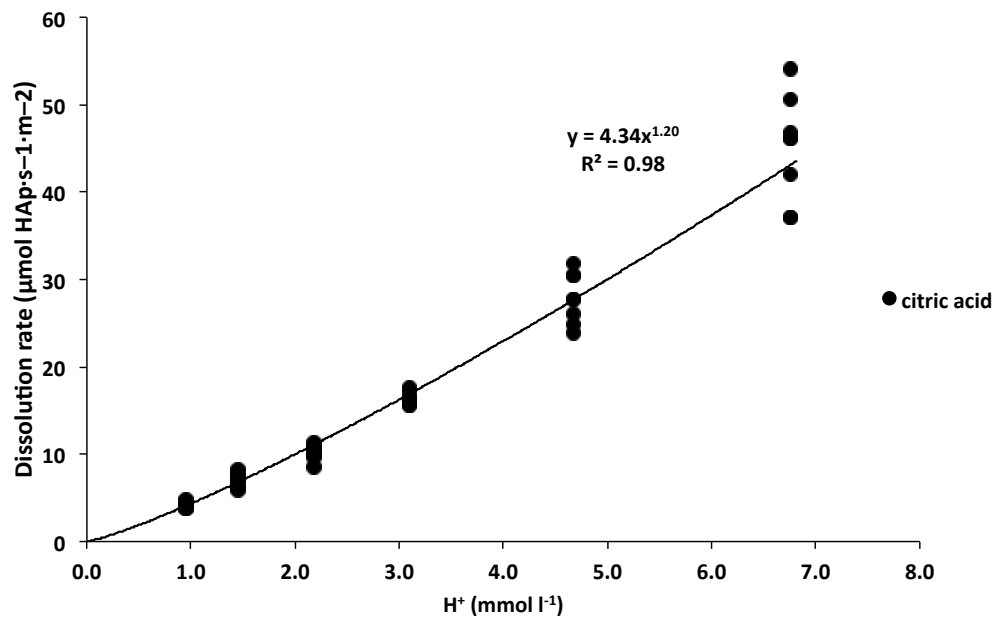


Figure 5: HAp-dissolution of enamel samples superfused with geometric dilution series of citric acid as function of initial H^+ -activity

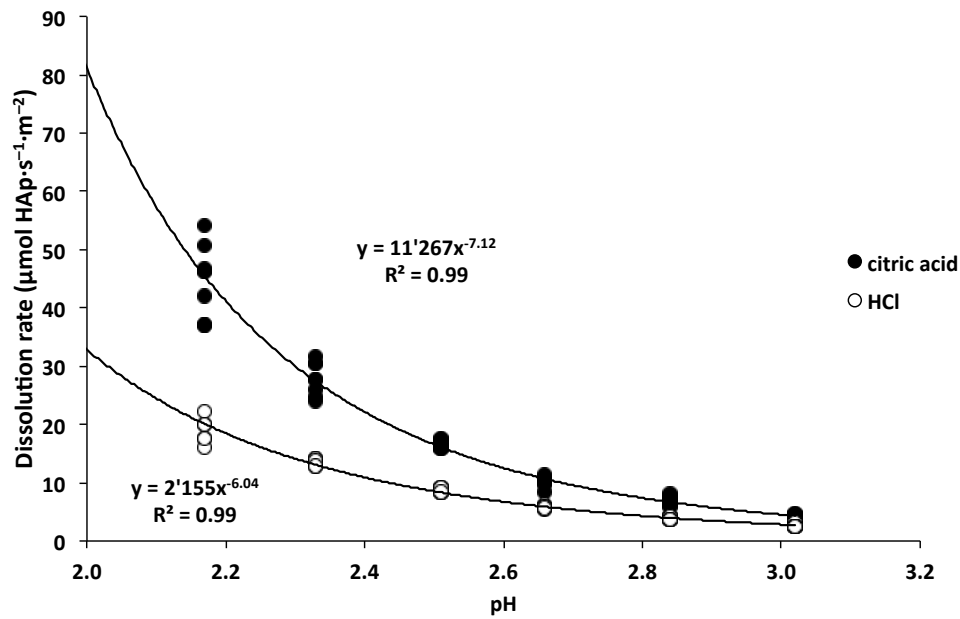


Figure 6: HAp-dissolution of enamel samples superfused with geometric dilution series of citric acid and HCl dilutions at the same pH-levels.

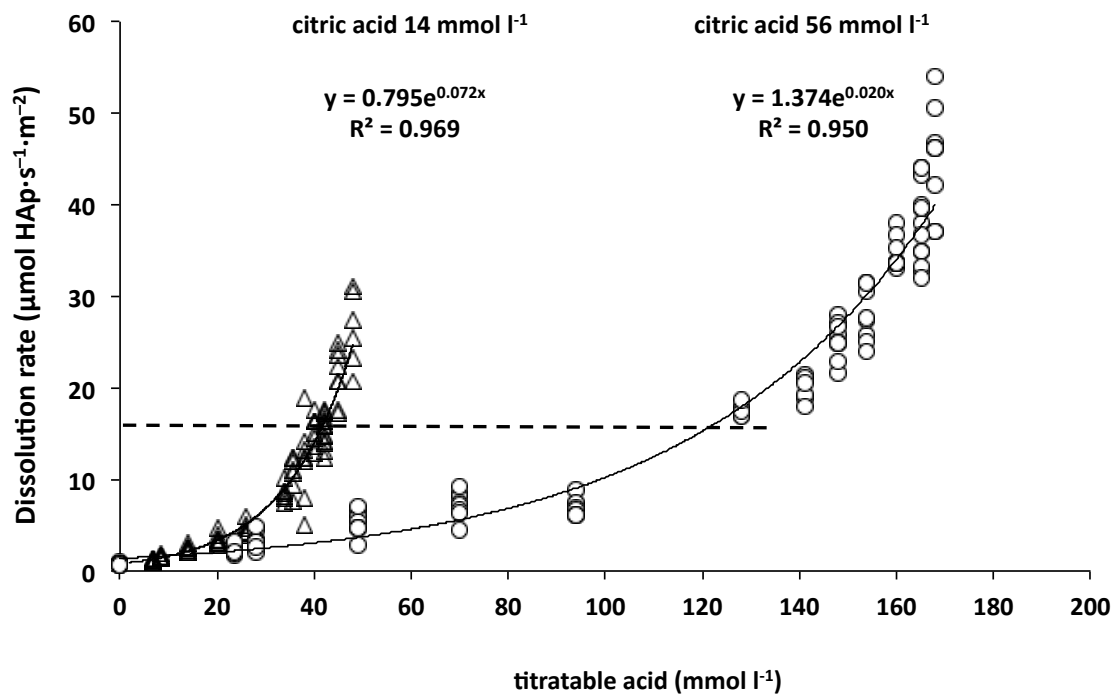


Figure 7: HAp-dissolution of enamel plotted against titratable acidity.

Enamel samples were superfused with two different concentrated citric acids (CA) adjusted to various titratable acidities (n=8).

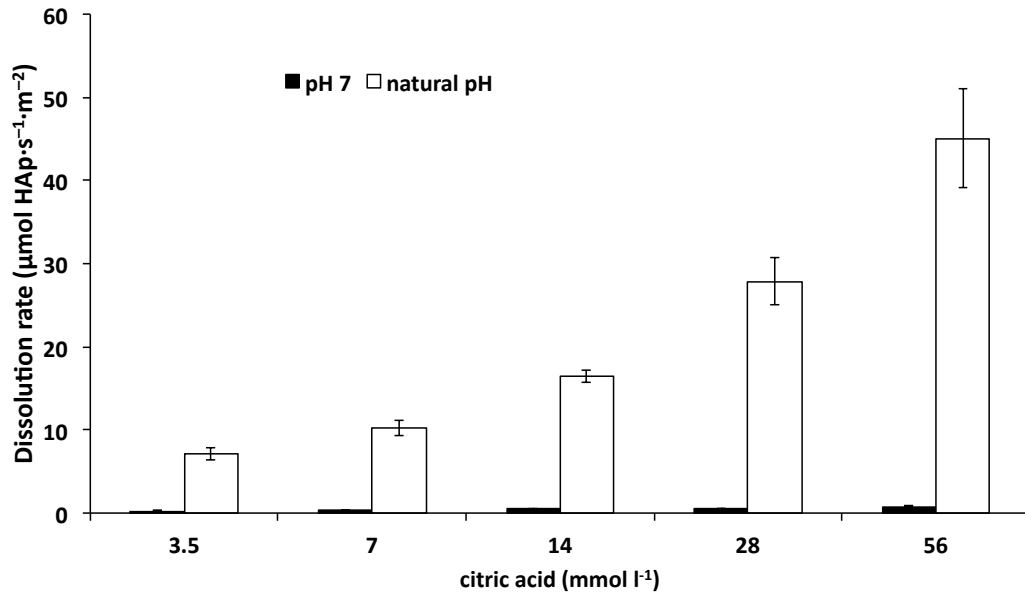


Figure 8: HAp-dissolution of enamel induced by natural (white columns) and neutralised (black columns) citric acid with different concentrations (mean \pm SD).

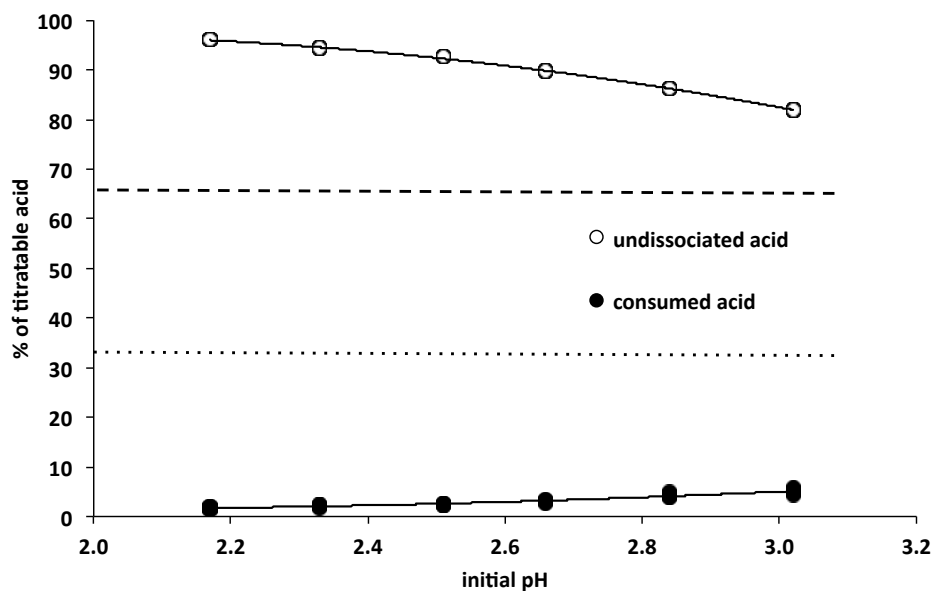


Figure 9: Percentage of un-dissociated acid and consumed acid (given as percentage of titratatble acid) of a geometric dilution series of citric acid with different initial pH during enamel superfusion.

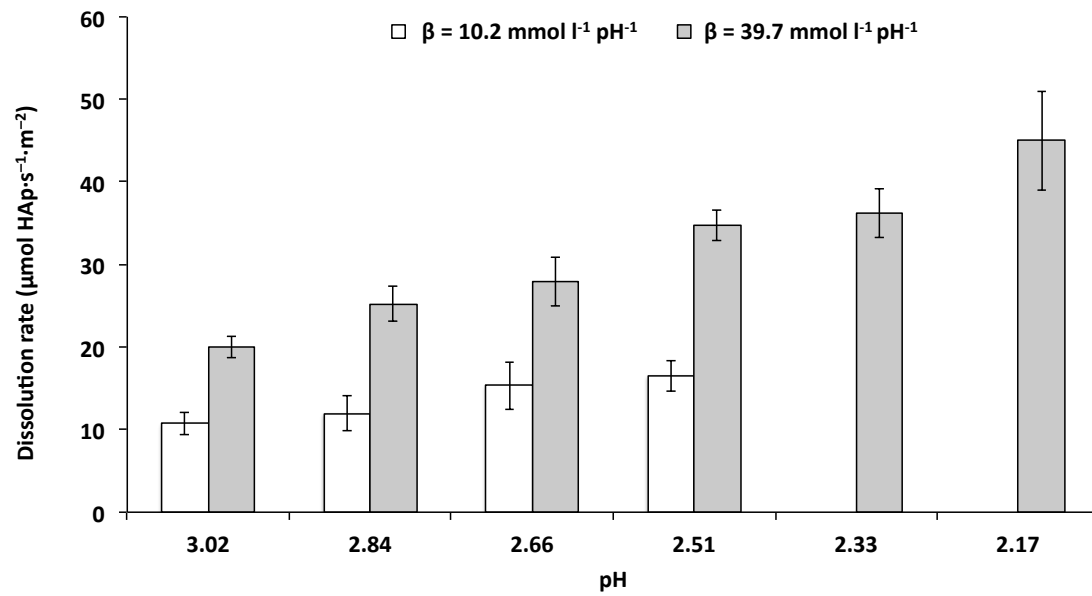


Figure 10: HAp-dissolution of enamel superfused with citric acid with two different buffer capacities (β) adjusted to the same pH-values (mean \pm SD).